

Optimization of Nanospray Voltage and Spray Stability: Impact on Chromatographic Peak Area for Analyte Quantitation

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Introduction

State-of-the-art liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis uses a constant electrospray (ESI) voltage for data acquisition. Modern qualitative and quantitative LC-MS/MS methods depend on highly efficient gradient elution chromatography. The changing chemical composition of mobile phase during gradient elution results in an inherent disconnect with single point ESI voltage optimization. A constant ESI voltage limits spray stability and compromises chromatographic peak area quantification, limiting total peak area and increasing peak area relative standard deviation (RSD). Using a nanospray source equipped with a digitally controlled stage and software for precise and reproducible emitter positioning for data acquisition we investigate the relationship between spray stability and data quality at flow rates of 200 nL/min, 500 nL/min and 1,000 nL/min (data not presented here).

Methods & Materials

Mass Spectrometer

- LTQ Linear Ion Trap (Thermo Scientific)
 - Full scan MS: 150 - 1,500 Da
 - Spray voltage: fixed per data file, variable across replicate injections as indicated
 - Analyte-specific targeted MS Scans
- DPV-550 Digital PicoView nanospray source (New Objective, Inc.)

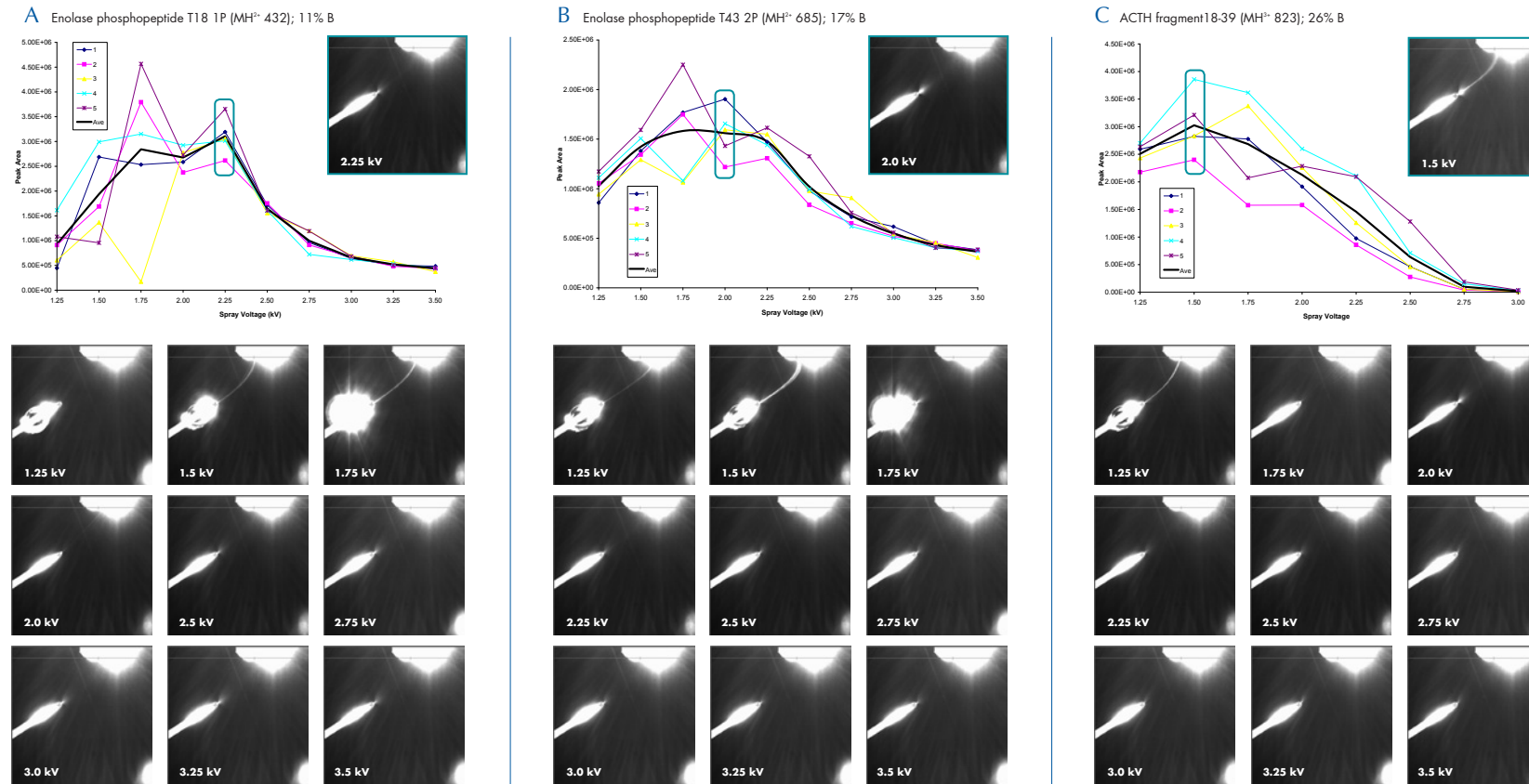
Chromatography

- Eksigent nanoLC-Ultra 2D plus
 - Flow rate: 200 nL/min, 500 nL/min
 - Mobile phase A: 0.1% Formic acid in water (JT Baker)
 - Mobile phase B: 0.1% Formic acid in acetonitrile (JT Baker)
 - Gradient: 30 minutes 2-50% B
- Column: PicoFrit column (360 μ m OD x 75 μ m ID x 15 μ m tip) slurry packed to 10 cm with BioBasic C18 (5 μ m, 300 \AA , C18, Thermo Scientific)
- HTC Pal autosampler (Leap Technologies)
 - 6-port injection valve (VICI Valco Instruments, Inc.), 1.0 μ L loop

Reagents

- Enolase phosphopeptide standard: 500 fmol/ μ L (Waters MassPREP)
- Bradykinin 1-7 fragment: 500 fmol/ μ L, MW 756.4 Da (Sigma-Aldrich ProteoMass)
- [Hyp3]-Bradykinin: 500 fmol/ μ L, MW 1076.2 (Sigma-Aldrich)
- Neurotensin: 500 fmol/ μ L, MW 1673.0 Da (Enzo Life Sciences)
- ACTH Fragment 18-39: 500 fmol/ μ L, MW 2465.2 Da (Sigma-Aldrich ProteoMass)
- Insulin chain B oxidized: 500 fmol/ μ L MW 3494.7 (Sigma-Aldrich ProteoMass)

FIGURE 2 200 nL/min.



Plot of extracted peak area for five replicate injections at ten different spray voltage settings for three different peptides. Compound specific peak area maximums are observed indicating gradient related optimal voltage settings for each. Peak area values can be correlated with the stability of the spray visualized in the photos. A) Enolase phosphopeptide T18 1P (MH⁺ 432) B) Enolase phosphopeptide T43 2P (MH⁺ 685) C) ACTH Fragment 18-39 (MH⁺ 823)

FIGURE 7 500 nL/min.

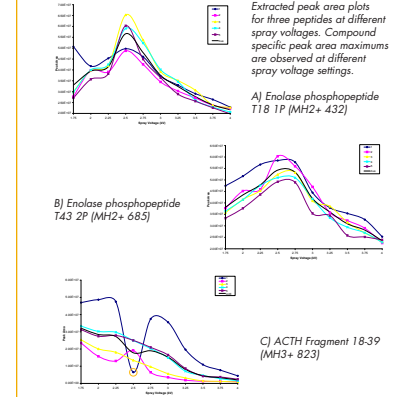
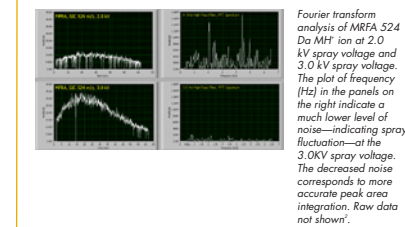
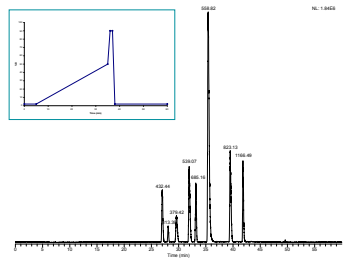


FIGURE 8 Frequency of spray stability



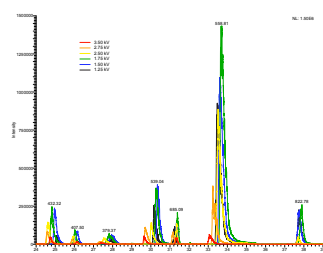
Fourier transform analysis of MRF 524 Da MH⁺ ion at 2.0 kV spray voltage and 3.0 kV spray voltage. The plot of frequency (Hz) in the panels on the right indicate a much lower level of noise—indicating spray fluctuation—at the 3.0 kV spray voltage. The decreased noise corresponds to more accurate peak area integration. Raw data not shown.

FIGURE 1 200 nL/min.



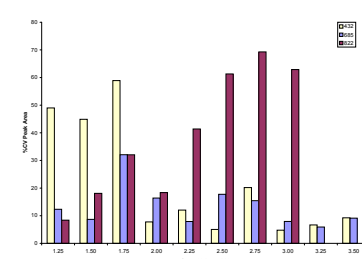
Base peak chromatogram of eight peptide mixture separated at 200 nL/min using a 30 minute gradient of 2 - 50% B.

FIGURE 3 200 nL/min.



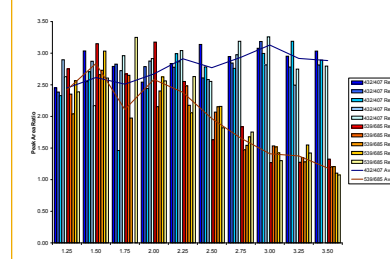
Overlay of six base peak chromatograms collected at different spray voltages. Changes in peak area can be observed for the absolute intensity scale.

FIGURE 4 200 nL/min. Change in peak area % CV for three peptides



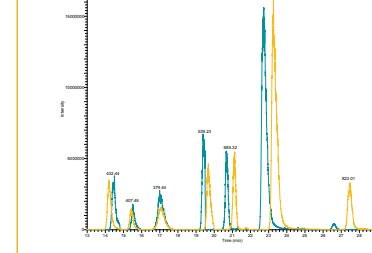
Extracted peak area %CV for three different peptides at different spray voltages. The lowest %CV value for each analyte directly correlates with the maximum peak area plots.

FIGURE 5 200 nL/min. Peak area ratio for 2 pairs of analytes



Peak area ratio of closely eluting peptides at different spray voltages.

FIGURE 6 500 nL/min. Sample degradation



Overlay of two base peak chromatograms of eight peptide mixture collected at 500 nL/min on two different days under identical conditions. Change in peak shape (increased tailing) and decrease in peak area highlights the change in sample composition as it degrades. Time between collection of data was 42 hours of storage at 4°C.

Results & Conclusions

Repetitive on-column injections at different (fixed) target ESI voltage settings were executed for a mixture of eight peptides with a wide range of elution composition. Plotting the chromatographic peak area for selected ion currents yields an apparent compound dependant response curve in which a total maximum value is observed. Peak area maximums were observed for spray voltage settings ranging from 2.25 kV to 1.5 kV at 200 nL/min. Image capture enabled by the Digital PicoView software program reveals a direct correlation between the observed spray mode, spray stability and chromatographic quality. Spray instability results in increased noise of the reconstructed chromatogram, increasing the uncertainty of peak area measurement. This instability is most apparent at the start and end of a gradient—high aqueous and high organic, where peak integration became impossible for the ACTH fragment MH3⁺ ion at spray voltages of 3.0 kV and greater. These observations are further supported statistically in the calculation of %CV for each analyte, achieving a minimal %CV value at 'optimal' spray conditions (i.e. voltage). Fourier transform spectrum analysis of the selected ion currents for individual analytes indicates the ion signal stability correlates with periodic (1-10 Hz) events associated with electrospray (voltage/flow rate) characteristics. The consistent relationship between spray frequency and data quality lends itself well to implementing an algorithm for gradient eluted voltage control for optimal peak area integration.

¹ Valaskovic, G.A., Murphy, J.P., Lee, M.S. "Automated Orthogonal Control System for Electrospray Ionization", J. Am. Soc. Mass Spectrom., 2004, 15, 1201-1215

² Valaskovic, Lee, M.S., Berg, A., "Evaluation of Nanospray Voltage and Spray Stability and their Impact on Chromatographic Peak Area", Poster presentation at 2011 Association of Biomolecular Resource Facilities, Sacramento, CA